H 1032

The Council For Tobacco Research–U.S.A., Inc.

110 East 59th Street
New York, N. Y. 10022
(212) 421-8885

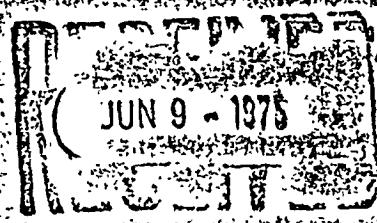
Application for Research Grant

(Use extra pages as needed)

Date: June 5, 1975

1. Principal Investigator (give title and degrees):

Olga Stein, M.D., Professor of Experimental Medicine



2. Institution & address:

Hebrew University-Hadassah Medical School
Jerusalem, Israel POB 1172

3. Department(s) where research will be done or collaboration provided:

Department of Experimental Medicine and Cancer Research and Department of Medicine

4. Short title of study:

Endothelial cell structure and function

5. Proposed starting date: January 1, 1976

6. Estimated time to complete: Three years

7. Brief description of specific research aims:

The specific aims of this research are related to the study of the glycoprotein residue which constitute a part of the endothelial cell surface coat. These glycoprotein residue will be identified with the help of specific lectins and attempts will be made to estimate their amount. The rate of renewal of the surface material glycoprotein under normal conditions will be studied and the effect of agents which modify various secretory processes will be determined. It is proposed also to determine the relation of surface material glycoproteins to the lipoprotein lipase that is active at the endothelial cell surface.

The information obtained under controlled conditions with normal tissue will serve as a basis for investigations in which the effect of various pollutants on the formation and maintenance of the endothelial surface coat can be studied.

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8. Brief statement of working hypothesis:

The endothelial cell surface can be visualized with ruthenium red, which apparently stains polysaccharide residues. The amount of the surface coat could vary under different conditions and hence a quantitation of the material will be attempted. The mode of formation of the material has not been elucidated and its rate of formation might be affected by agents which modify transport of secretory products or membrane precursors. The endothelial cell surface has been considered as the site of action of lipoprotein lipase, and hence a possible relation between this enzyme and the surface glycoproteins will be sought.

9. Details of experimental design and procedures (append extra pages as necessary)

For the identification of glycoprotein residues in the surface of endothelial cells several lectins will be used which bind to specific sugar residues. Concanavalin A, wheat germ agglutinin phytohaemagglutinin and ricin for α-D-mannose, α-D-glucose, α-D-fructose, (Con A) N-acetyl glucosamine, N-acetylgalactosamine and d-galactose, respectively. In order to render them identifiable by electron microscopy the lectins will be complexed to peroxidase (1) or ferritin (2) and purified to remove unbound markers. Alternately the lectin will be visualized after it had been bound to the cell surface by reacting it with either horseradish peroxidase followed by the demonstration of enzyme activity or with hemocyanin, which binds specifically to ConA residues (3) and can be identified in shadow cast replicas. The lectins will be radioiodinated with I^{125} and purified from Sephadex G 50 columns (3).

The tissues to be studied will include the aorta, coronary arteries and myocardial capillaries of rats and mice and after the establishment of the techniques the most relevant experiments will be repeated in rabbits. In order to preserve the intactness of the endothelium the tissues will be studied after in vivo fixation with 1% paraformaldehyde, a procedure which was shown not to affect the quantity or specificity of binding of concanavalin A (3). Following exposure to the lectin its visualization will be carried out on tissues prepared for electron microscopy. When peroxidase will be used to reveal the presence of lectins, sections will be prepared with a Smith-Farquhar tissue sectioner and peroxidase activity will be visualized with the Karnovsky procedure. Following post-fixation with osmium tetroxide, dehydration and embedding, ultrathin sections will be examined with the Philips EM 300 electron microscope. To study the coronary arteries and heart capillaries use will be made of coronary perfusion to introduce the lectins into the vascular lumen. Preliminary experiments have shown that binding of concavalin A to the endothelial cell surface introduced by perfusion of coronary circulation can be demonstrated at the surface of heart capillaries both by radioautography and peroxidase activity. For the validation of the specificity of binding, appropriate sugar derivatives specific for each lectin will be used to prevent binding or to dissociate the bound lectin. In order to minimize endocytosis when unfixed hearts will be studied the perfusion will be carried out at 10°-18°C. The quantitation of the binding sites will be attempted with the help of

A. radioautography at the light microscope or electron microscope level, after exposure of sections to radioautographic emulsion
B. counting of ferritin on properly enlarged electron micrographs
C. in the case of the aorta use will be made also of hemocyanin. After exposure to hemocyanin the tissue will be fixed and dehydrated in ethanols and dried, preferably with critical point drying. Replicas will be prepared, cleaned and examined under the electron microscope.

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9. continued

To study renewal of the surface coat glycoproteins use will be made of ^{3}H-fucose, which is known to be incorporated into glycoproteins (4). The rats or mice will be injected with the labeled precursor and tissues prepared for radioautography at 5-120 min time intervals. The distribution of the radioautographic grains will be determined over the cell body and the cell surface. In order to learn about the effect of agents, which modify the transport of secretory products, on the rate of renewal of surface glycoproteins, the animals will be pretreated with colchicine or vinblastin for 1-4 h prior to labeling with ^{3}H-fucose.

To study the relation between lipoprotein lipase and the surface glycoproteins use will be made of the various lectins. In order to determine whether the sugar residues of the membrane bound enzyme are exposed, hearts will be perfused with radioiodinated lectins and then lipoprotein lipase will be released into the perfusat by introduction of heparin (5). In preliminary experiments it has been learned that concanavalin A does not interfere with the measurement of enzyme activity.

1. N.K. Gonatas and S. Avrameas. J. Cell Biol. 59:436, 1973.
2. G.L. Nicolson and S.J. Singer. Proc. Natl. Acad. Sci. 68:942, 1971.
3. S.B. Smith and J.P. Revel. Develop. Biol. 27:439, 1972.
J.Z. Rosenblith, T.E. Ukena, H.H. Yin, R.D. Berlin and M.J. Karnovsky, Proc. Nat. Acad. Sci. 70:1625, 1973.
4. J. Bennet, C.P. Leblond and H. Haddad. J. Cell Biol. 60:258, 1974.
5. E. Atkin and H.C. Meng. Diabetes 21:149, 1972.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The work on the project will be carried out in the laboratory for electron microscopy of the department of experimental medicine and cancer research and in the lipid research laboratory of the department of medicine. Both laboratories are located in the same building and have adequate facilities for both the morphological and biochemical studies. These include electron microscope facilities, ultramicrotomes, dark rooms, scintillation counters, cold rooms and animal rooms.

11. Additional facilities required:

Not applicable

10035402/27

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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12. Biographical sketches of investigator(s)

Olga Stein, principal investigator

1952 M.D. from Hebrew University Hadassah Medical School, Jerusalem
1953-1958 Department of Pathology, Hebrew University Hadassah Medical School
1958-present Department of Experimental Medicine and Cancer Research Med. School, Jerusalem
1960-1961 and 1967-1968 Visiting Scientist, The Rockefeller University, New York
1969 Associate Professor, Experimental Medicine, Hebrew University
1974 Professor of Experimental Medicine, Hebrew University
1975 Visiting Professor, University of California, San Diego

Tova Chajek, coinvestigator

1971 M.D. from Hebrew University Hadassah Medical School, Jerusalem
1971-present Department of Medicine B, Hadassah University Hospital, Jerusalem, and in Lipid Research Laboratory
1972 Instructor in Medicine
1974 Lecturer in Medicine

Yechezkiel Stein, coinvestigator

1952 M.D. from Hebrew University Hadassah Medical School, Jerusalem
1953-present Department of Medicine B, Hadassah University Hospital, Jerusalem
1963 Associate Professor of Medicine
1965 Head of Lipid Research Laboratory
1960-1961 and 1967-1968 Visiting Scientist, Rockefeller University, New York
1969 Professor and Head of Department of Medicine
1975 Visiting Professor, Rockefeller University, N.Y. and University of California, San Diego

13. (1) CIBA Found. Symp. 12:165, 1973
(2) J. Cell Biol. 62:90, 1974
(3) Biochim. Biophys. Acta 360:205, 1974
(4) Biochim. Biophys. Acta 380:106, 1975
(5) Biochim. Biophys. Acta 380:127, 1975

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R: REDACTED MATERIAL

14. First year budget.

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
O. Stein, M.D.	30%	-
T. Chajek, M.D.	25%	-
Y. Stein, M.D.	10%	-
Technical		
Technician for electron microscopy, M.Sc.grade	100%	REDACTED
Technician for biochemical study, M.Sc. grade	100%	

*includes social benefits

Sub-Total for A REDACTED

B. Consumable supplies (by major categories)

Lectins (Con A, WGA, Ricin)	1,000
Chemicals, including osmic acid	2,000
Glassware	1,000
Isotopes (^{3}H Fucose, ^{125}I)	1,000
Electron microscopy supplies	1,000

Sub-Total for B 6,000

C. Other expenses (itemize)

Animals + upkeep	2,000

Sub-Total for C 2,000

Running Total of A + B + C 26,000

D. Permanent equipment (itemize)

None

Sub-Total for D

E. Indirect costs (16% of A+B+C) E 3,900

15. Estimated future requirements. Total request 29,900

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	6,500	2,500	4,200		33,350
Year 3	R	7,000	3,000	4,800		36,800

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Lipid metabolism by smooth muscle cells in culture	U.S.-Israel Binational Science Foundation (Grant to Y. Stein)	$15,000	8/1/75-7/31/76

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Olga Stein

Signature Olga Stein Date 6/14/75

Telephone 212 (Area Code) 4111 (Number) 475 (Extension)

Checks payable to

Mailing address for checks

Responsible officer of institution

Typed Name

Title

Signature Date

Telephone (Area Code) (Number) (Extension)

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